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Hepatic Sarcocystosis in a Striped Dolphin (*Stenella coeruleoalba)* From the Spanish Mediterranean Coast

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ABSTRACT: Fatal hepatic sarcocystosis was diagnosed in a striped dolphin (*Stenella coeruleoalba*) from the northeastern Spanish Mediterranean coast based on pathologic findings and the microscopic and ultrastructural characteristics of the intralesional parasite. Main gross lesions were icterus, subcutaneous hemorrhages, and hepatic congestion. The most prominent microscopic lesions consisted of severe acute multifocal to coalescing necrotizing hepatitis with cholestasis and intralesional protozoa. There was severe chronic pancreatitis with generalized distension of pancreatic ducts by hyaline plugs and adult trematodes. Only asexual stages of the protozoa were found. The parasite in the liver divided by endopolygeny. Schizonts varied in shape and size. Mature schizonts had merozoites randomly arranged or budding peripherally around a central residual body. Schizonts were up to 22 μm long, and merozoites were up to 6 μm long. Ultrastructurally, merozoites lacked rhoptries. This parasite failed to react by immunohistochemistry with anti–*Toxoplasma gondii*, anti–*Neospora caninum* and anti–*Sarcocystis neurona* antibodies. The microscopic and ultrastructural morphologies of the parasite were consistent with *Sarcocystis canis*, so far described only from animals in the Unites States. The life cycle and source of *S. canis* are unknown. The present report of *S. canis*–like infection in a sea mammal from Spain indicates that the definitive host for this parasite also exists outside of the United States.

Sarcocystis species generally have a 2-host predator–prey life cycle with an asexual cycle in herbivores and a sexual cycle in carnivores (Dubey et al., 1989). *Sarcocystis* species are generally host specific for their intermediate host. A new group of *Sarcocystis* parasites (e.g., *S. neurona* and *S. canis*) were discovered in the past decade with unusual host range and life cycles. *Sarcocystis neurona* causes central nervous system disorders in horses and a variety of other mammals (Dubey et al., 2001) considered to be aberrant hosts because only schizonts are found in these hosts and are confined to the central nervous system. Opossums are the definitive hosts shedding sporocysts of *S. neurona;* cats, and armadillos can act as intermediate hosts with sarcocysts in their muscles (Dubey, Saville et al., 2000; Cheadle et al., 2001; Tanhauser et al., 2001).

Sarcocystis canis is related to *S. neurona* but its life cycle is unknown (Dubey and Speer, 1991). Only the schizont stage is known and is confined mostly to the liver. It produces fatal hepatitis in dogs, chinchillas, bears, horses, sea lions, and Hawaiian monk seals (Dubey and Speer, 1991; Mense et al., 1992; Rakich et al., 1992; Zeman et al., 1993; Garner et al., 1997; Yantis et al., 1998; Davis et al., 1999; Trasti et al., 1999). Until now, all reports of *Sarcocystis*-associated hepatitis were from the United States. In the present report, *S. canis*–associated hepatitis is documented in a dolphin from the Spanish Mediterranean coast.

An 80-kg, 215-cm-long, adult female striped dolphin (*Stenella coeruleoalba*) was found stranded alive in the northeastern Spanish Mediterranean coast in March 1997 and died shortly thereafter. At necropsy, samples of lung, heart, liver, stomach, spleen, pancreas, adrenal, kidney, urinary bladder, tongue, brain, skeletal muscle, skin, mammary gland, and lymph nodes were fixed in neutral buffered 10% formalin, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin. Formalin-fixed liver was routinely processed for transmission electron microscopy. Immunohistochemistry was performed to screen for antigens of *Toxoplasma gondii*, *Neospora caninum*, and *S. neurona* in liver and brain, as previously described (Juan-Sallés et al., 1997; Lindsay and Dubey, 1989; Dubey and Hamir, 2000). Similarly, the brain and lymph node were tested for phocine distemper virus (PDV) using a monoclonal antibody against the F protein of PDV (Trudgett et al., 1991) as previously described (Domingo et al., 1992). This antibody cross-reacts with dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV).

Gross findings consisted of icterus, obvious in the subcutaneous tissue, aorta, pulmonary vessels, renal pelvis, and cerebrospinal and eye anterior chamber fluid. There were also multiple dorsolateral subcutaneous hemorrhages and hepatic congestion. Several macerated fish were present in the first stomach compartment, and multiple parasitic nodules with focally ulcerated mucosa were observed in the glandular and pyloric compartments. Other gross findings included mesenteric lymphadenopathy, pale white streaks on the cut surface of the tongue, as well as a 4-cm, air-filled, bullous cavity with numerous nematodes attached to its inner surface and adhesions of the left lung to the parietal pleura. Plerocercoid cysts of *Phyllobothrium delphini* and *Monorygma grimaldii* were present in the blubber of the genital area and in the abdominal cavity, respectively, and 1 *Xenobalanus* sp. was observed in the fluke.

Microscopically, the most prominent findings affected the liver and pancreas. Hepatic lesions consisted of an acute necrotizing hepatitis characterized by random foci of lytic and coagulative hepatocellular necrosis associated with mixed inflammatory infiltrates and periportal infiltrates composed mainly of plasma cells, macrophages, lymphocytes and a few eosinophils (Fig. 1). There was marked intracanalicular and hepatocellular cholestasis and hepatic congestion. Numerous protozoa were found in the cytoplasm of hepatocytes surrounding areas of necrosis (Figs. 1, 2). Only asexual stages were seen. Both immature and mature schizonts were seen. The earliest schizonts were up to 5 by 9 μm and contained a large nucleus with a prominent nucleolus (Fig. 2A, B). The parasite was undergoing endopolygeny, a process in which the nucleus becomes enlarged and multilobulated before dividing into numerous merozoites (Fig. 2C). Mature schizonts varied in shape and size, occupied most of the hepatocyte cytoplasm, and displaced the cell nucleus peripherally (Fig. 2C, G). Schizonts were up to 22 μm in diameter and contained a variable number of merozoites; 35 merozoites are present in 1 plane of section in Figure 1F. Merozoites were haphazardly arranged or budded peripherally, occasionally around a central residual body (Fig. 2C, D). Merozoites varied in length and shape. The longest merozoites were 6 μm long in section (Fig. 1F). Individual merozoites were rarely seen within hepatocytes or free in the foci of necrosis (Fig. 1B, C).

Ultrastructurally, schizonts appeared to lie directly in the cytoplasm of hepatocytes, with no visible parasitophorous membrane. Merozoites contained numerous micronemes, a conoid, and a centrally placed nucleus, but no rhoptries. Schizonts and merozoites failed to react with *T. gondii*, *N. caninum*, and *S. neurona* antibodies and were not observed in hematoxylin and eosin–stained sections from the other tissues.

In this dolphin, chronic pancreatitis was prominent and characterized by a marked, generalized distension of pancreatic ducts and adjacent acini by plugs of a pale eosinophilic amorphous material, small noncalcified calculi, or both, as well as mild periductal and interlobular fibrosis with periductal mononuclear inflammatory infiltrates constituted mainly of lymphocytes and plasma cells; some ducts were also distended with trematodes with spines in their cuticle. Multifocally in the interstitium, there were aggregates of lymphocytes and plasma cells, often with intralesional trematode eggs. There was also pancreatic multifocal to coalescing acinar atrophy and degeneration. Additional microscopic

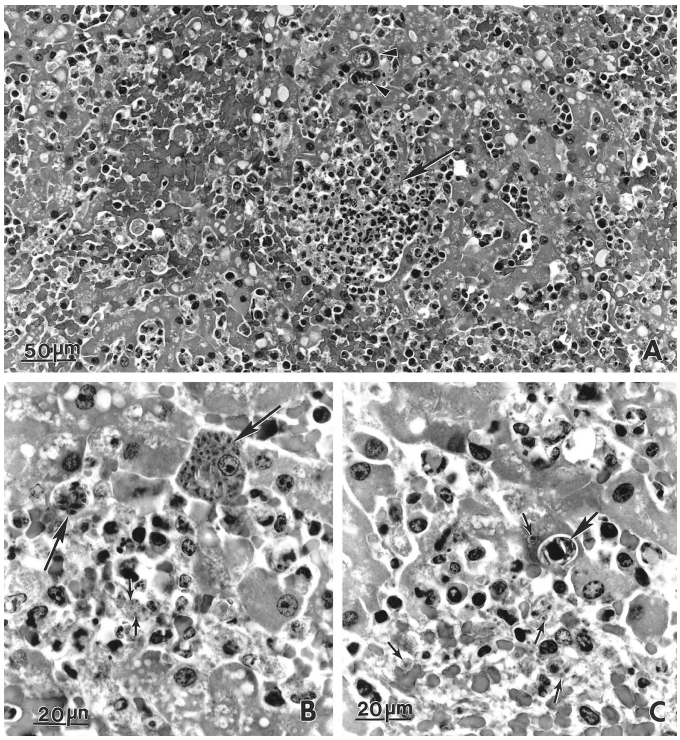

FIGURE 1. Multifocal to coalescing necrotizing hepatitis in a dolphin infected with a *Sarcocystis canis*–like parasite. Hematoxylin and eosin stain. (**A**) One central focus of lytic hepatocellular necrosis (arrow) associated with infiltrates of lymphocytes, plasma cells, macrophages, and few eosinophils around single and groups of hepatocytes. Arrowheads point to 2 schizonts. (**B, C**) Foci of necrosis with infiltrates of lymphocytes, plasma cells, macrophages, and few eosinophils. Note schizonts (arrows) at the periphery of the lesion. Individual merozoites (arrows) are difficult to recognize among degenerating host cells.

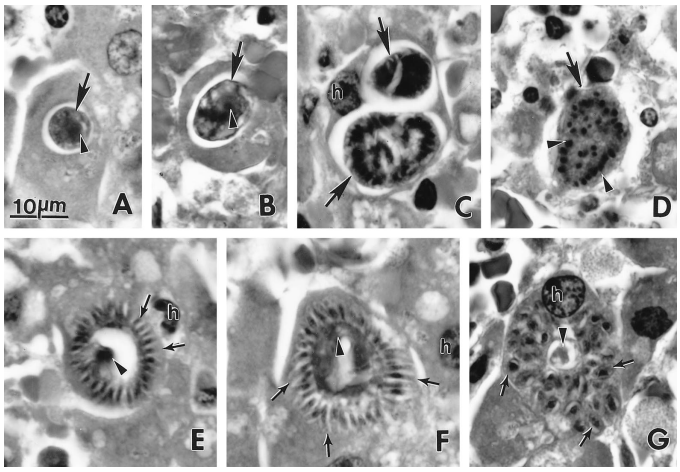

FIGURE 2. Schizongonic stages of a *Sarcocysts canis*–like parasite in liver of the dolphin. Hematoxylin and eosin stain. Bar = 10 µm and applies to all figures. h, host cell nucleus. (**A, B**) Young schizont (arrows) with individual nucleus with a nucleolus (arrowheads). (**C**) Two schizonts (arrow) in 1 hepatocyte. The nucleus in the bigger schizont has become lobed. (**D**) A degenerating schizont (arrow) with multilobed nucleus (arrowheads). (**E**) Merozoites arranged at the periphery and attached by a rim of host cells. Note a small residual body (arrowhead). (**F**) Schizont with a large residual body (arrowhead) and slender merozoites (arrows). (**G**) Merozoites (arrows) dispersed in cytoplasm of the hepatocyte. Note a small residual body (arrowhead).

findings were mild multifocal pyogranulomatous and necrotizing adrenalitis; multifocal granulomatous panniculitis with ceroid pigment in the cytoplasm of foamy macrophages, multinucleated giant cells, and necrotic adipocytes; mild degenerative myopathy of the tongue; marked systemic lipopigment deposition (neurons, skeletal fibers in the tongue, and muscular layers of the urinary bladder); mild mononuclear perivascular infiltrates of lymphocytes and plasma cells in the brain; granulomatous gastritis with intralesional *Pholeter gastrophilus*; and focal granulomatous mesenteric lymphadenitis with intralesional unidentified parasite eggs. Immunohistochemistry for DMV was negative.

The death of this dolphin was attributed to *Sarcocystis*–associated hepatitis that apparently caused hepatic failure as a consequence of the massive hepatic necrosis and intrahepatic cholestasis. Icterus and subcutaneous hemorrhages were attributed to impaired liver function, together resulting in failure of multiple organ systems and death. Fatal hepatic sarcocystosis has not been previously described in dolphins. Hepatic lesions and the intralesional parasite are similar to those described in hepatic sarcocystosis in a horse, a sea lion, a chinchilla, a Hawaiian monk seal, an American black bear, and 2 polar bears (Mense et al., 1992; Rakich et al., 1992; Zeman et al., 1993; Garner et al., 1997; Yantis et al., 1998; Davis et al., 1999) and also in *S. canis*–associated hepatitis reported in fatal visceral and neural sarcocystosis of dogs (Dubey and Speer, 1991; Trasti et al., 1999). In the sea lion with hepatic sarcocystosis, muscular sarcocysts were also present, but no clear relationship between the 2 stages was found (Mense et al., 1992). No sarcocysts were observed in this dolphin, but an extensive sampling of skeletal muscle was not performed. *Sarcocystis* infection has been occasionally reported in cetaceans with muscular sarcocysts, in striped dolphin, pilot whale (*Globicephala melaena*), and sperm whale (*Physeter catadon*) (Cowen, 1966; Owen and Kakulas, 1967; Dailey and Stroud, 1978). However, hepatic sarcocystosis has not been previously reported in any cetacean species. The interest in *Sarcocystis* infections of marine mammals is growing; *S. neurona*–like infection recently has been described in captive sea otters (*Enhydra lutris*) (Rosonke et al., 1999; Lindsay et al., 2000, 2001; Miller et al., 2001) and wild pacific harbor seals (*Phoca vitulina richardsi*) (Lapointe el al., 1998). In this dolphin, the parasite divided by endopolygeny, thus excluding from the differential etiologic diagnosis *Toxoplasma* and *Neospora*, which divide by endodyogeny, and merozoites lacked rhoptries, which is characteristic of *Sarcocystis* species (Dubey et al., 1989). Additionally, the parasite failed to react to *T. gondi*, and *N. caninum* antibodies.

The hepatic parasite found in this dolphin was diagnosed as a *S. canis*–like organism based on morphology. Schizonts had a central residual body, which is prominent in *S. canis* schizonts (Dubey and Speer, 1991). *Sarcocystis canis* schizonts resemble schizonts of *Frenkelia* (Dubey, Clark, and Yantis, 2000), but this organism is not known to infect large mammals (Garner et al., 1997). It also resembles *S. neurona*; both *S. canis* and *S. neurona* typically cause necrosis, as observed in this case, whereas lesions in other *Sarcocystis* species are usually inflammatory. Additionally, *S. canis* and *S. neurona*–like organisms usually do not infect endothelial cells, whereas other *Sarcocystis* species often do (Rakich et al., 1992). However, *S. neurona* is known to be limited to the central nervous system (Dubey, Davis et al., 1991), and the *Sarcocystis* species found in this dolphin failed to react to *S. neurona* antibodies. Although this parasite closely resembles *S. canis* and *S. canis*–like organisms causing hepatic sarcocystosis, further studies would be necessary to clarify their relationship.

The life cycle and source of infection of *S. canis* are unknown. It is speculated that the hepatic phase of *S. canis* may be a stage of *Frenkelia* (Dubey, Clark, and Yantis, 2000). *Frenkelia* and *Sarcocystis* are structurally and phylogenetically related parasites, and *Frenkelia* has been considered a synonym of *Sarcocystis* (Mugride et al., 1999). The present report of *S. canis*–like infection in a sea mammal from Spain indicates that the definitive host for this parasite also exists outside of the United States.

Based on knowledge of other *Sarcocystis* species, the definitive host of this organism is likely a carnivore or scavenger, but the life cycles of unknown or unstudied *Sarcocystis* species may vary somewhat from those of studied species (Dubey et al., 1989). Striped dolphins inhabit strictly oceanic waters, feed mainly on cephalopods and fish (Perrin et al., 1994), and also drink sea water (Worthy, 1990). Dolphins could

merely be aberrant hosts for *S. canis*, similar to the role of horses for *S. neurona*. Chronic pancreatitis with ductal plugs, calculi, and trematodes was a striking finding in this dolphin and may have caused exocrine pancreatic insufficiency. Trematodes have been reported to cause similar lesions in other cetaceans (Howard et al., 1983). This dolphin was in a poor body condition but had a stomach full of food; moreover, the presence of ectoparasites (*Xenobalanus* sp.) are indicative of physical debilitation. Panniculitis with ceroid and systemic lipopigment deposition is suggestive of vitamin E deficiency, which is a common complication of fat malabsorption syndromes in human beings, such as those occurring in exocrine pancreatic insufficiency (Shael et al., 1986; Nakaruma and Takeuchi, 1997). A possible relationship between hepatic sarcocystosis and chronic pancreatitis cannot be ruled out, because vitamin E deficiency is associated with impaired immunity in animals and man (Meydani et al., 1995), and impairment of immunity can increase host susceptibility to protozoal infections (Cawthorn and Speer, 1990).

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